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RENAL C4D DEPOSITION IS A MARKER OF HEMATOPOIETIC STEM CELL TRANSPLANT (SCT)-ASSOCIATED THROMBOTIC MICROANGIOPATHY (TMA)

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C4d is a marker of classical complement activation by tissue specific antibodies (abs) or endothelial damage. Currently, peritubular capillary (PTC) C4d deposition aids in the diagnosis (dx) of ab mediated kidney transplant rejection (AMR). An uncontrolled case series reported PTC and glomerular C4d staining in patients with SCT-TMA (Mii et al, Path Int 2011). Expanding on these findings, we hypothesized that renal tissue from patients with SCT-TMA would more frequently show C4d deposition compared to controls.

Methods: Retrospective analysis of all renal biopsy and autopsy specimens from pediatric patients undergoing SCT at our institution. Using histological criteria alone, patients were divided into TMA and non-TMA (control) groups. C4d staining was performed by immunohistochemistry on formalin-fixed, paraffin-embedded tissue and was evaluated on arterioles, PTCs, glomeruli, and tubular basement membranes. Specifically, rabbit anti-human C4d polyclonal ab was applied to sections and C4d was independently graded (diffuse: 50-100%; focal: 5-50%; rare: 1-5%; negative: 0%) by two pathologists, blinded to each subject's underlying dx.

Results: 20 total specimens were identified (19 subjects). Of these, 8 specimens (7 subjects) had histologic evidence of TMA (5 biopsy, 3 autopsy). 1 TMA subject had both a biopsy and autopsy with similar findings, therefore only the autopsy findings are included. The 12 SCT recipient autopsies without TMA served as controls. Diffuse and focal arteriolar C4d staining was far more common in TMA specimens compared to controls (71.4% versus 8.3%, p = 0.01). PTC C4d staining was present in just under half of TMA samples and was absent in controls (p = 0.04). Glomerular C4d staining was similar in both groups and tubular basement membrane staining was rare.

Conclusions: This is the first report of arteriolar C4d deposition in SCT-TMA, implicating localized complement fixation due to as yet unidentified abs or ab-independent direct endothelial damage in the pathogenesis of TMA. PTC C4d staining was found only in TMA, while glomerular staining was non-specific as it was similar in both groups. Although the preferential arteriolar C4d staining is incompletely understood, it may identify a renal site of injury, explaining the dramatic hypertension often seen in TMA. Future research should assess if patients with SCT-TMA and renal C4d deposition will benefit from therapies currently used to treat kidney transplant AMR.

Table. C4d Deposition in TMA

	Histology proven TMA (n = 7)	Control (n = 12)	p-value*
Pathology Results			
Arteriolar C4d+	5/7 (71.4%)	1/12 (8.3%)	0.01
PTC C4d+	3/7 (42.9%)	0/12 (0%)	0.04
Glomerular capillary C4d+	4/7 (57.1%)	10/12 (83.3%)	0.30
Tubular basement membrane C4d+	2/7 (28.6%)	0/12 (0%)	0.12
Clinical Results			
Age (years)	3.6 (1.7-4.5) [0.6-4.9]	3.2 (1.4-17.1) [0.9-28.1]	0.55
Gender (male)	3/7 (42.9%)	8/12 (66.7%)	0.38
Specimen day post-SCT	237 (43 - 631) [19 - 2281]	172 (78 - 253) [9 - 887]	0.50
Malignancy	5/7 (71.4%)	2/12 (16.7%)	0.03
Immunodeficiency	1/7 (14.3%)	9/12 (75.0%)	0.03
Bone marrow failure	1/7 (14.3%)	1/12 (8.3%)	0.03
Allogeneic	2/7 (28.6%)	11/12 (91.7%)	0.01
Autologous	5/7 (71.4%)	1/12 (8.3%)	0.01
GVHD in those at risk (allogeneic)	1/2 (50%)	3/11 (27.3%)	1.00
Viral infection	4/7 (57.1%)	4/12 (33.3%)	0.38
Dialysis	6/7 (85.7%)	5/12 (41.7%)	0.15

*from Fisher's exact test for categorical variables and Wilcoxon Rank sum for continuous variables; data expressed as n (%) or median (25th-75th percentile) [range].

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NAïVE CD19-SPECIFIC T CELLS EXHIBIT SUPERIOR PROLIFERATION AND POTENTIAL FOR ADOPTIVE IMMUNOTHERAPY

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T cells in human peripheral blood can be characterized as naïve (T_N), central memory (T_{CM}) and effector memory (T_{EM}). We and others seek to define the best cellular substrate for genetic modification and expression of tumor-specific immunoreceptors, such as a CD19-specific chimeric antigen receptor (CAR) for adoptive transfer in pediatric and adult patients after hematopoietic stem cell transplantation. Naïve-derived effector cells ($T_{EFF[sup]N[/sup]}$) are an attractive T-cell population for adoptive immunotherapy as they confer improved anti-tumor activity in a mouse (pmel-1 tumor) model. Therefore, we determined the relative frequency of T_N ($CD62L^+CD45RA^{neg}$), T_{CM} ($CD62L^+CD45RA^{neg}$), T_{EM} ($CD62L^{neg}CD45RA^{neg}$) and T_{EMRA} ($CD62L^{neg}CD45RA^+$) subsets in peripheral blood mononuclear cells (PBMC) from normal healthy donors (n = 21) and observed an abundance of circulating T_N cells compared to T_{CM} ($p<0.001$), and T_{EM} ($p<0.05$). To determine if we could retrieve CAR^+ $T_{EFF[sup]N[/sup]}$ and propagate them ex vivo to clinically-appealing numbers, PBMC were sorted into three T-cell subsets and genetically modified by electroporation to express a CD19-specific CAR using the Sleeping Beauty (SB) DNA plasmid system. Our artificial antigen presenting cells (aAPC) were then used to numerically expand CD19-specific T cells in a CAR-dependent manner. We observed CD19-specific T cells in all subsets, with total cell numbers higher in $T_{EFF[sup]N[/sup]}$ than $T_{EFF[sup]CM[/sup]}$ or $T_{EFF[sup]EM[/sup]}$. These increased cell numbers were explained by the enhanced proliferative capacity of $T_{EFF[sup]N[/sup]}$. Although specific cytolytic ability was diminished for CAR^+ $T_{EFF[sup]N[/sup]}$, in contrast to the other T-cell subsets, they secreted more IFN-γ in response to CD19. These data suggest that T_N in PBMC can be selectively electroporated and propagated using our current platforms (SB and aAPC system) for generating clinical-grade CAR^+ T cells.

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EXPOSURE OF EARLY TRAINEES TO BONE MARROW TRANSPLANT LEADS TO HIGHER BMT PHYSICIAN RECRUITMENT

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The National Marrow Donor Program (NMDP) projects the need for allogeneic unrelated blood and marrow transplants (BMT) in the United States is 10,000 per year. While the NMDP is preparing to facilitate that number by 2015, there are a number of barriers to meeting this need including recruiting additional health care personnel including BMT providers. To learn how best to recruit BMT physicians, we sought to understand why practicing BMT physicians chose to enter BMT, and why others did not. We conducted a web-based survey amongst Pediatric Hematology/Oncology (PHO) and BMT physician providers and trainees to determine the factors influencing their decision to choose or not choose a career in BMT. There were 259 respondents (48% male, 74% of Caucasian origin); 94 identified as BMT physicians, 112 as PHO physicians and 53 as PHO trainees. PHO and BMT providers spent an average of 53% in clinical